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IN THE ADULT RAT

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A TECHNIQUE FOR THYMECTOMY IN THE ADULT RAT

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ABSTRACT

An improved technique for surgical removal of the thymus in the adult rat was developed. Essentially the procedure consisted of forming an aperture to the left of the midline and below the clavicle which minimized excessive trauma and permitted easy aspiration of the thymus. Innovar-Vet was used to produce anesthesia. The animals recovered fully within 2 hours after surgery. Wound clips were removed within 14 days. The survival rate for all rats was 80 percent.

I. INTRODUCTION

Thymectomy of the adult laboratory animal is a useful adjunct to the study of certain immunological processes, such as cell-mediated immunity and graft rejection. Techniques for thymectomizing adult mice,^{2,3,8,12} hamsters¹¹ and rats^{1,4,7,9,10,13} have appeared in the literature. However, a detailed description of thymectomy in the adult rat is not available. Thymectomy of the adult rat is difficult to perform due to the close association of the thymus with surrounding connective tissue.

Commonly used anesthetic agents such as Nembutal or ether have been associated with high mortality rates because of difficulties in dosage regulation of Nembutal and impaired respiration due to hypersecretion of mucus following ether anesthesia. Therefore, we sought a fast acting substitute which would assure a quick recovery and obviate undesirable side effects. Innovar-Vet,* an analgesic marketed for dogs and recently used in several other species,^{5,6} causes negligible mortality and does not have the side effects of ether or Nembutal. This paper details a technique for thymectomy of adult rats using Innovar-Vet.

II. MATERIALS AND METHODS

Lewis rats (Lew/f Mai), free of chronic murine respiratory virus (CMRV) and weighing 125-150 g, were given subcutaneous injections of 0.04 mg/kg of atropine sulfate (Eli Lilly and Company, Indianapolis, Indiana) 10 min prior to an intramuscular injection of 0.1 ml/kg of Innovar-Vet. This compound is marketed as an analgesic; however, it produces a state of anesthesia which develops within 10 min after injection and lasts about 2 hours.

* Innovar-Vet^R contains 0.4 mg fentanyl and 20 mg droperidol per ml.
Pitman-Moore, Inc., Washington Crossing, New Jersey.

The thoracic and submandibular areas were shaved with animal clippers. A depilatory was not required. The animal was placed in a supine position upon a dissecting board with its head toward the operator. The legs were secured to the operating surface by means of pins and stretched rubber bands placed around the junction of the radio-ulnar and carpal regions and of the tibiofibular and tarsal regions. The neck and chest were moistened with 70 percent ethanol. A longitudinal midline incision was made to the left of the midline through the skin and superficial fascia from the level of the angle of the mandible to the fourth rib (Figure 1).

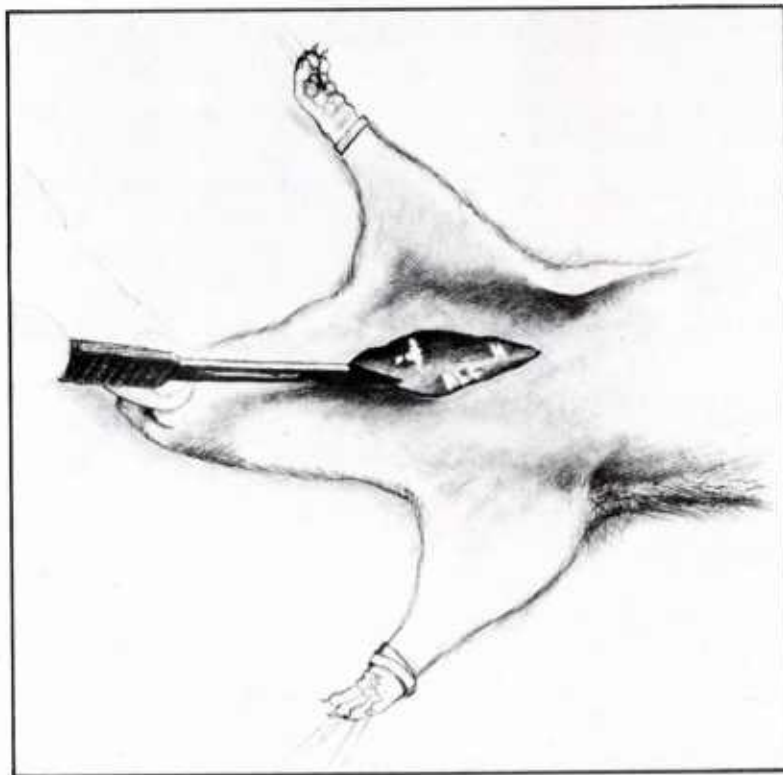


Figure 1. The initial incision is made through the skin and superficial fascia from the level of the angle of the mandible to the fourth rib

Blunt forceps (12 cm) were used to free the skin from the underlying muscle for ease in closure. The pectoralis muscle was transected to the left of the midline starting just below the clavicle and proceeding no further than the second rib. An incision of this size and at this level facilitated closure and avoided damage to the major blood vessels. The second and third ribs were visualized and clipped and reflected exposing the thymus, which appeared as a glistening organ anterior to the heart. A suction tube was immediately inserted into the chest cavity to aspirate the thymus. Aspiration was assisted by using a toothless iris forceps to divide all areolar tissue connections between the intact gland and the surrounding tissue (Figure 2). Approximately 45 sec were available for aspiration of the thymus. If the thoracic cavity was open for longer than 60 sec, fatal pncumothorax inevitably occurred. If the suction procedure required

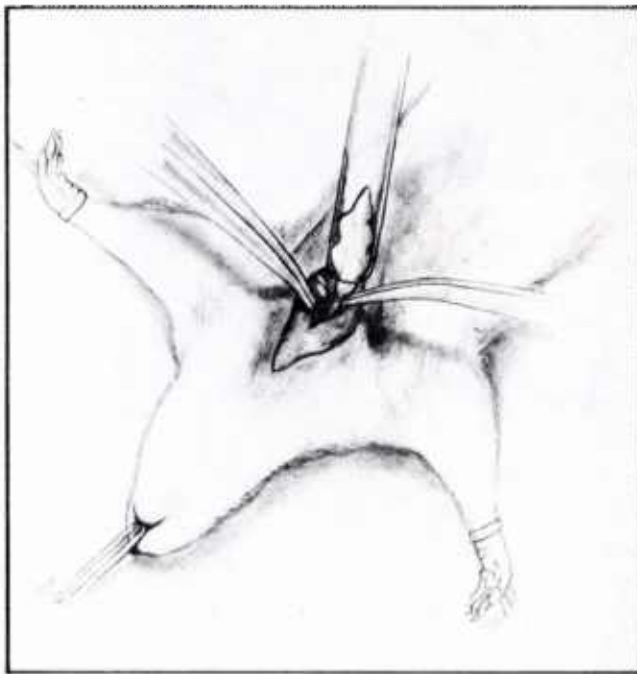


Figure 2. Aspiration of the thymus

longer than 45 sec at any particular time, the cavity was closed manually or with 12-cm toothed forceps until the animal resumed normal breathing. A rubber bulb fitted with a two-way valve and a 4-inch piece of Tygon tubing served as a respirator for use in those animals suffering respiratory distress.

Care was taken to avoid injury to the right and left superior vena cava. Some bleeding was expected and was not overly detrimental to recovery. After visual inspection ascertained that no thymic remnants were present, air was expressed from the thoracic cavity by massage directed from the diaphragm toward the head. The thoracic walls were apposed with two 12-cm dressing forceps by the assistant while the operator closed the chest with 4-0 surgical gut medium chromic sutures and a 3/8 circle taper needle. The skin was closed with 9-mm stainless steel wound clips. The animals were then allowed to recover in a cage under a heat lamp at 35°C for 1-2 hours. Within 30 min after closure, the animals regained their normal motor control; the anesthetic effect of the Innovar-Vet subsided completely after 2 hours. Wound clips were removed 14 days after surgery.

The vacuum necessary for aspiration of the thymus was provided by a suction flask attached to a pump or other source capable of providing a pressure equivalent to 16 inches of mercury. Aspiration of the thymus was done with a glass tube which allowed visualization of the organ as it was withdrawn. The glass tube was 16 cm long with an o.d. of 6 mm and an i.d. of 4 mm. A 150° elbow bend was made at the midpoint to facilitate handling, and the ends were fire-polished to avoid trauma. When this procedure was followed precisely as described above, a survival rate of 80 percent was obtained.

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